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Influence of C/N ratios on the Heterotrophic activity of model brackish water systems

Theivasigamani Anand, Gunalan Balakrishnan, Pandurangan Padmavathy, V.Rani, Anand Chandran

Theivasigamani Anand
Assistant Professor, Institute of Fisheries Technology, Tamil Nadu Fisheries University, Nagapattinam, Tamil Nadu - 620011, India
Email: oceananand@yahoo.co.in

Gunalan Balakrishnan
Assistant Professor, Centre of Advanced Studies in Marine Biology Faculty of Marine Sciences, Annamalai University, Parangipettai, Tamil Nadu - 608502, India
Email: aquagunal@gmail.com

Pandurangan Padmavathy
Assistant Professor, Department of Fisheries Environment, Fisheries College & Research Institute, Tuticorin, Tamil Nadu - 628 008, India
Email: padmarengan1968@yahoo.co.in

V.Rani
Assistant Professor
Maritech Research and Extension Centre, Tharuvaikulum, Tuticorin, Tamil Nadu - 628 008, India
Email: ranidarshini@gmail.com

Anand Chandran
Assistant Professor,
Institute of Fisheries Technology, Tamilnadu Fisheries University, Nagapattinam, Tamil Nadu -620011, India
Email: cee_anand@yahoo.com

Correspondence:
Theivasigamani Anand
Assistant Professor, Institute of Fisheries Technology, Tamil Nadu Fisheries University, Nagapattinam, Tamil Nadu - 620011, India
Email: oceananand@yahoo.co.in

ABSTRACT
The heterotrophic bacteria play a major role in pond mineralization. In the present investigation the effects of different C/N ratios and different carbon sources on the population and activities of heterotrophs in model brackish water systems were investigated under laboratory conditions. In brackish water and brackish water sediments irrespective of the carbon source, the C/N ratio would significantly influence the highest concentration of ammonia registered. In brackish water the C/N ratio somewhat lower than 20:1 was observed to be optimal for the highest activity and population of heterotrophs irrespective of the carbon source. Among the different carbon sources tried glucose ranked first in ammonia uptake and also supporting the heterotrophic population followed by sucrose and starch. Due to different C/N ratios, and kinds of carbon amendments in brackish water sediments, ammonia assimilation by heterotrophs was observed to suppress the nitrification process. The population of heterotrophic nitrite oxidizers showed significant ($P<0.01$) positive correlation with the level of available nitrite both in sediment and water of all treatments. So the present investigation confirmed that the enhancement of C/N ratios with suitable carbon source may control the concentration of ammonia and nitrite in aquaculture systems.

Keywords: Heterotrophs, Carbon amendment, Ammonia oxidizers, Aquaculture systems, CN ratio.

1. Introduction

In natural aquatic ecosystems heterotrophic microbial biomass contribute to ten percent of the growth of detritivorous fishes [12, 39]. Such heterotrophic microbial biomass and exudates represent a major nutritional source as energy and essential amino acids [40]. Recent research works carried out on heterotrophic bacteria have confirmed that under nitrogen limiting condition or relative enrichment of carbon, heterotrophs can quantitatively remove ammonia and nitrate enriched in the water [8, 4, 6]. In brackish water shrimp culture systems also ammonia and nitrate might function as the surplus source of nitrogen [13] for heterotrophic activity. This observation assumes importance in shrimp culture systems where ammonia, nitrite the product of ammonia oxidation pose problem to shrimps under culture [31]. Conversion of toxic concentrations of ammonia and unused nitrate into heterotrophic biomass warrants much scientific concern in semi-intensive culture systems that depend to an appreciable extent on basic food of quality protein. Perhaps, utilization of ammonia and nitrate by heterotrophs in carbon enhanced condition may serve as a better measure to maintain non toxic concentrations of ammonia and nitrite rather than using nitrifying bioremediations. In waste water treatment also such biological removal of nitrate and biosynthesis of protein will assume more significance than the conventional denitrification process. Apart from these the carbon amendment technique has been reported to eliminate pathogens, by creating severe competition between heterotrophs and pathogens under nitrogen limiting condition [15]. Thus, manipulation of C/N ratio of sediment appears to be a highly promising technique in improving water quality conditions and controlling of pathogen in aquaculture systems.

1.1 The major objectives of the present study were

1. To investigate the relationship existing between C/N ratio and heterotrophic activity in model brackish water systems
2. To identify the impact of C/N ratio on nutrient regeneration process
3. To ascertain the impact of various sources of carbon on the heterotrophic activity

1.2 Carbon to nitrogen ratio (C/N ratio) and manipulation of C/N ratio

Low and high bacterial activity was observed at C/N ratios [26] less than 10 and greater than 20 respectively. The critical carbon to nitrogen ratio of freshwater aquaculture systems used to range between 20 and 25 [11]. C/N ratio was an important factor in soil fertility [34] as an indicator of the rate of decomposition of organic matter by heterotrophy. Close relationship existed between the organic matter and nitrogen content of soils and demonstrated that C/N ratio of soils was fairly constant [14] and also suggested that C/N ratio in soil organic matter was important as it created keen competition among microorganisms for available soil nitrogen when residues with high C/N ratio were added to soil.

Deficiency in any element required for heterotrophic nutrition could limit decomposition [27, 23]. To assimilate the nutrient in limitation the heterotrophs would compete even with phytoplankters [34]. The substrate and microbial biomass C/N ratios and the activity of biomass decide the degradation of organic carbon and organic nitrogen [7]. Thus the C/N ratio of substrates manipulated by adding the nutrient in limitation to increase the heterotrophic activity [4, 14, 22, 41, 42].

1.3 Organic carbon enrichment

There is a critical C/N ratio in which microbial and chemical steady state is maintained. At C/N ratios above this critical value, heterotrophic and nitrifying population becomes nitrogen limited [43]. When C/N ratio is below the critical level the carbon enrichment should be made to enhance the heterotrophic activity [44].

In intensive aquaculture systems the organic nitrogen accumulates due to left out and undigested feed, excretion and manuring [5, 28]. Thus, due to surplus nitrogen the prevalence of toxic inorganic nitrogen species viz. ammonia and nitrite also evidenced by many workers [17, 32, 24]. Carbon enhancement in such systems would lead to utilization of nitrogen pool by heterotrophic bacterial biomass [6, 8, 4, 29]. These workers explained that the addition of carbonaceous substrates to culture systems would trigger nitrogen uptake from water to produce microbial cell protein. The microbial biomass increased with the addition of glucose along with nitrogen and phosphorus and micro nutrients to soil and the build up and decline of microbial biomass [22] could be correlated with immobilization and release of mineral nitrogen.

2. Materials and Methods

In the present study on the effects of various C/N ratios and carbon amendments with different sources of carbon (glucose, sucrose and starch) on heterotrophic activity was carried out in model brackish water systems.

Laboratory models of brackish water systems were developed to ascertain the impact of various C/N ratios and carbon sources on the heterotrophic activity of sediments and water.

2.1. Preparation of Sediments for the experiment

Sediment samples meant for the experiment were collected from estuarine systems to ascertain the ratio of carbon to nitrogen. All the collected sediment samples were dried and sieved (80 meshes per inch) to remove coarse particles. Estuarine sediments having an approximate C/N ratio of 5:1 were identified. As in most of the sediments there was a relative decrement in nitrogen thus, suitable quantity of urea was added to fix the exact ratio as 5:1 in the dried samples.

2.2. Carbon amendment and modification of C/N ratio

In the present study the influence of C/N ratios affected by different carbon sources on heterotrophic activity was investigated in detail. Three types of carbon sources, such as glucose, sucrose and starch representing monosaccharide, disaccharide and polysaccharide were used for effecting carbon amendment and altering C/N ratio of the sediments to three different fixed values i.e. 20:1, 50:1, 100:1. Urea application was effected when the C/N ratio of the sediment was to be fixed as 1:1. The carbon and nitrogen amended brackish water sediments with different C/N ratios were placed in experimental tanks of 25 liters capacity. Suitable quantity of unfiltered aged seawater was added to the sediment to affect a sediment water ration of 1:5. The depth of the sediment in all the experimental tanks did not exceed 1cm. The sediment-water mixture was aerated continuously till the end of the experiment. The sediment was mostly in suspended condition near the site of aeration. The dissolved oxygen (DO) concentration in brackish water systems fell below 1ml/*l* in higher C/N ratios.

2.3. Chemical and microbiological analyses of water and sediment samples

Initial and weekly collection of water and sediment samples was carried out and the collected samples were immediately analyzed. Ammonia nitrogen, nitrite nitrogen and nitrate nitrogen contents of the water samples were assessed adopting the procedures of FAO [21]. Modified Winkler's titration method was adopted to estimate dissolved oxygen. The exchangeable fractions of ammonia nitrogen, nitrite nitrogen and nitrate nitrogen were leached out following the method described [26] and were analyzed adopting the methods of FAO [21]. The collected sediment samples were preserved and processed as per the procedures detailed by Ramadhas and Santhanam [35]. The sedimentary organic carbon (SOC) and sedimentary organic nitrogen (SON) were analyzed adopting the procedures of Ramadhas and Santhanam [35].

2.4. Microbiological examination of water and sediment samples

Microbiological analyses were carried out for water and sediment samples collected from model brackish water systems. All the samples were collected aseptically in presterilized containers. The total heterotrophic counts (THC) were estimated following the procedures of [3]. To enumerate the population of heterotrophic ammonia and nitrite oxidizers the ATCC media 438 and 480 modified method [37] which were solidified by bacteriological grade (Himedia) agar were used. Spread plating technique was used for the inoculation and the plates were incubated for 48 hours at ambient temperature. The formation or destruction of nitrite around the colonies (as tested by azo dye method) indicated the oxidation of ammonia to nitrite and nitrite to nitrate, respectively.

3. Results

3.1. C/N ratio amendment and nitrogen assimilation in model brackish water systems

Values of ammonia, nitrite and nitrate recorded in model brackish water systems with different C/N ratios affected by the application of urea, glucose, sucrose and starch are given in Tables 1, 2, 3 and 4. Unlike in C:N ratios of 1:1 and 5:1, addition of glucose and sucrose to effect different C/N ratios invariably resulted in hydrogen sulfide production, during the different later phases of the experiment. In the case of starch, hydrogen sulfide production appeared at the end of eighth week in all the three ratios investigated in water and sediment. In the case of glucose application, increase in ratio was observed to expedite hydrogen sulfide production. In the case of higher C/N ratios (50:1 and 100:1) total disappearance of ammonia, nitrite and nitrate or hydrogen sulfide production could be recorded in both water and sediment beyond the experimental period of three weeks.

Sucrose application moderately supported the utilization of ammonia and nitrate by heterotrophs and expedited production of hydrogen sulfide could be recorded only at the end of the third week in the ratio of 100: 1. In higher ratios of C: N total disappearance of nitrate could be recorded at the end of the third week. In comparison, glucose application was more effective than the other two sources of carbon used for enhancing C: N ratio. Disappearance of nitrite and nitrate prior to hydrogen sulfide formation well documented the operation of denitrification process. Starch was not observed to pose any problem up to seven weeks, as the uptake rates of ammonia and nitrate were moderate.

Two way ANOVA analysis [18] confirmed that in brackish water the highest concentration of ammonia recorded varied significantly ($P<0.05$) among the four different ratios tested (Table 12). In the sediment also C/N ratio significantly ($P<0.05$) influence the highest concentration of ammonia recorded (Table 13). However, the different sources of carbon used for amendment did not significantly influence the highest value of ammonia recorded in both water and sediment of model brackish water systems.

Log value of ammonia concentration and normal values of C/N ratios or log values of ammonia concentration and log values of C/N ratios were correlated suitably to rectify the curvilinear slope as a straight line slope. In all the three treatments both in water and sediment of brackish waters significant negative correlation was observed between the highest values of ammonia recorded and C/N ratio (Table 14).

Table 1: Mean values of ammonia, nitrite and nitrate recorded in the model brackish water systems with different C/N ratios amended with urea

Days	Water (W) / Sediment (S)	1:1			5:1		
		µg.at.N/l					
		N_1	N_2	N_3	N_1	N_2	N_3
Initial	W	23.91	13.24	11.10	22.56	11.26	3.71
	S	71.14	15.36	9.41	25.71	8.94	2.80
7th day	W	121.36	15.31	13.27	56.31	9.31	5.83
	S	151.97	27.21	11.86	56.23	13.78	4.23
14th day	W	1943.46	26.14	19.36	2155.83	26.46	7.81
	S	2563.84	31.29	14.17	378.93	11.23	7.84
21st day	W	6422.01	111.99	53.23	682.20	3.18	20.80
	S	7618.90	89.73	17.23	591.78	15.92	11.84
28th day	W	4712.10	91.20	61.78	465.91	20.23	18.61
	S	3986.72	56.17	22.14	713.62	6.27	10.93
35th day	W	3564.21	76.40	72.31	276.40	18.76	21.11
	S	1763.41	21.130	25.03	523.10	13.48	14.26
42nd day	W	2170.31	53.40	78.90	151.21	16.13	23.24
	S	813.14	15.27	22.79	412.37	10.03	16.31
49nd day	W	976.43	45.23	69.31	76.78	19.38	25.18
	S	563.23	17.43	24.26	279.81	5.06	19.23
56th day	W	431.25	31.27	72.34	54.21	18.16	27.29
	S	426.37	12.03	25.78	117.19	6.16	20.73

N1-Ammonia nitrogen, N2- Nitrite nitrogen, HP- hydrogen sulfide produced, N3- Nitrate nitrogen, BDL - Below Detectable Limit

Table 2. Mean values of ammonia, nitrite and nitrate recorded in the model brackish water systems with different C/N ratios amended with glucose

Days	Water (W) Sediment(S)	20:1			50:1			100:1		
		µg.at.N/l			µg.at.N/l			µg.at.N/l		
		N_1	N_2	N_3	N_1	N_2	N_3	N_1	N_2	N_3
Initial	W	23.50	7.23	3.26	1.37	2.71	23.36	22.93.	5.36	0.36
	S	39.73	10.12	2.78	43.86	12.93	1.76	140.17	13.28	2.61
7th day	W	79.36	5.21	5.12	106.23	2.76	4.28	60.50	11.87	1.93
	S	105.61	13.73	4.12	241.83	21.86	3.81	123.01	23.71	3.01
14th day	W	109.93	12.74	7.38	120.85	9.28	1.23	15.23	13.92	3.19
	S	196.20	23.17	9.13	456.90	15.27	5.32	39.26	31.16	4.63
21st day	W	396.65	18.32	6.31	21.29	3.2	BDL	HP	HP	HP
	S	661.27	21.76	12.21	BDL	11.2	BDL	HP	HP	HP
28th day	W	15.38	5.08	2.73	HP	HP	HP	HP	HP	HP
	S	50.86	11.26	1.13	HP	HP	HP	HP	HP	HP
35th day	W	1.21	2.13	0.81	HP	HP	HP	HP	HP	HP
	S	5.61	1.61	BDL	HP	HP	HP	HP	HP	HP
42nd day	W	0.72	0.16	BDL	HP	HP	HP	HP	HP	HP
	S	1.72	0.08	BDL	HP	HP	HP	HP	HP	HP
49nd day	W	HP	HP	HP	HP	HP	HP	HP	HP	HP
	S	HP	HP	HP	HP	HP	HP	HP	HP	HP
56th day	W	HP	HP	HP	HP	HP	HP	HP	HP	HP
	S	HP	HP	HP	HP	HP	HP	HP	HP	HP

N_1- Ammonia nitrogen, N2 ₋ Nitrite nitrogen, N_3- Nitrate nitrogen, BDL - Below Detectable Limit HP - hydrogen sulfide produced

Table 3: Mean values of ammonia, nitrite and nitrate recorded in the model brackish water systems with different C/N ratios amended with sucrose

Days	Water (W) Sediment(S)	20:1			50:1			100:1		
		µg.at.N/I			µg.at.N/I			µg.at.N/I		
		N_1	N_2	N_3	N_1	N_2	N_3	N_1	N_2	N_3
Initial	W	22.94	1.94	0.711	23.15	8.74	2.31	22.76	11.12	0.93
	S	51.71	9.28	2.31	63.26	9.32	1.61	55.79	16.13	2.31
7th day	W	134.91	15.62	3.86	341.13	16.21	4.79	103.21	19.74	3.93
	S	175.86	20.16	5.60	576.84	27.69	3.72	354.16	23.78	4.32
14th day	W	429.00	31.27	6.93	125.98	13.17	5.26	32.91	16.48	4.51
	S	617.16	29.23	7.03	232.33	21.28	6.37	107.23	10.21	5.04
21st day	W	156.73	40.39	BDL	1.86	10.21	1.03	BDL	5.26	BDL
	S	217.26	51.71	BDL	112.06	3.36	0.59	16.10	1.01	BDL
28th day	W	5.86	27.25	BDL	BDL	5.21	BDL	HP	HP	HP
	S	56.17	42.39	BDL	BDL	0.13	BDL	HP	HP	HP
35th day	W	0.93	1.12	HP	HP	HP	HP	HP	HP	HP
	S	BDL	3.26	HP	HP	HP	HP	HP	HP	HP
42nd day	W	HP	HP	HP	HP	HP	HP	HP	HP	HP
	S	HP	HP	HP	HP	HP	HP	HP	HP	HP
49nd day	W	HP	HP	HP	HP	HP	HP	HP	HP	HP
	S	HP	HP	HP	HP	HP	HP	HP	HP	HP
56th day	W	HP	HP	HP	HP	HP	HP	HP	HP	HP
	S	HP	HP	HP	HP	HP	HP	HP	HP	HP

N_1- Ammonia nitrogen, N2 - Nitrite nitrogen, N_3- Nitrate nitrogen, BDL - Below Detectable Limit HP - hydrogen sulfide produced

Table 4: Mean values of ammonia, nitrite and nitrate recorded in the model brackish water systems with different C/N ratios amended with starch.

Days	Water (W) Sediment(S)	20:1			50:1			100:1		
		µg.at.N/I			µg.at.N/I			µg.at.N/I		
		N_1	N_2	N_3	N_1	N_2	N_3	N_1	N_2	N_3
Initial	W	24.01	13.17	2.76	46.73	23.92	3.71	23.17	2.73	1.22
	S	97.41	21.24	5.03	48.16	8.19	4.15	46.73	5.26	2.73
7th day	W	161.03	20.26	5.21	57.98	11.23	5.24	156.17	8.26	2.13
	S	159.23	31.78	6.17	160.74	16.84	3.97	215.63	11.71	3.16
14th day	W	311.26	21.13	7.43	1560.21	27.34	4.63	560.01	12.78	4.23
	S	98.71	43.12	9.23	405.16	39.27	5.41	297.45	8.93	5.07
21st day	W	1760.35	23.57	8.59	1023.12	52.91	6.01	205.71	19.16	6.73
	S	524.76	35.26	11.72	326.34	18.13	7.23	215.17	10.96	7.85
28th day	W	1211.23	40.56	13.12	750.23	43.26	8.43	149.1	15.21	8.92
	S	409.38	29.71	16.41	179.73	21.23	9.41	176.21	23.17	11.26
35th day	W	796.29	39.27	11.26	302.14	58.79	11.29	45.21	29.76	10.13
	S	217.94	33.12	18.41	206.21	22.16	6.21	78.39	11.93	13.36
42nd day	W	303.14	45.89	5.17	136.73	21.13	13.16	11.13	21.23	12.94
	S	96.81	11.27	3.26	53.62	11.07	0.27	39.23	6.19	15.17
49nd day	W	76.23	12.14	0.79	25.10	9.64	BDL	5.23	24.93	BDL
	S	19.756	4.26	BDL	29.84	3.26	BDL	11.16	BDL	BDL
56th day	W	HP	HP	HP	BDL	HP	HP	HP	HP	HP
	S	HP	HP	HP	HP	HP	HP	HP	HP	HP

N_1- Ammonia nitrogen, N2 - Nitrite nitrogen, N_3- Nitrate nitrogen, BDL - Below Detectable Limit HP - hydrogen sulfide produced

3.2. C/N amendments and the population of heterotrophic microbial population:

The numbers of heterotrophs (THC) occurring in water and sediment of model brackish water systems with different C/N ratios effected by the application of urea, glucose, sucrose and starch are given in Tables 5, 6 and 7. In brackish water model systems irrespective of the source of carbon the highest mean THC were mostly (except starch application) encountered in the C/N ratio of 100:1. Thus, the mean heterotrophic counts were observed to be decided by the C/N of both sediment and water. In the case of starch application quite strikingly, both the sediment and water of model brackish water systems registered the lowest mean THC in the C/N ratio100:1 followed by the ratio 50:1. Apparently, the restricted heterotrophic activity in the starch injected system was responsible for the anaerobic condition for a prolonged period.

3.3. C/N amendments and the population of heterotrophic ammonia and nitrite oxidizers in model brackish water systems

Numbers of heterotrophic ammonia and nitrite oxidizers recorded in the water of the model brackish water systems with different C/N ratios affected by urea and the three different sources of carbon are furnished in Tables 8 to 11. When a comparison is made between the substrate available and the respective population of nitrifiers it became apparent that ammonia oxidizers not occur in abundance as expected from the level of their substrate. However, population of nitrite oxidizers was observed to be substrate specific. However, nitrite oxidizers were more substrate specific and their population could be correlated with nitrite concentration in the sediment and water of brackish water model systems (Table 15).

3.4. Experiment on nutrient limitation

When ammonia and nitrate started to disappear from the water of model fresh water and brackish water systems a solution of ammonium chloride (10 µg. at N/ml) alone, a mixture of nitrogenous nutrient and phosphate (2 µg.at.N + 1 µg.at. $P0_4$-P/ml) and a mixture of biochemically important trace metals (iron, manganese, molybdenum, cobalt, zinc, with each metal in a strength of 0.1 µg/ml) with ammonia and phosphate were individually added/added in the combination of nitrogen, phosphorus and trace metals to identify the limiting nutrient. The results obtained confirmed that none of the trace metals or phosphate limited the growth of heterotrophs and injection of ammonia triggered the heterotrophic population as indicated by active ammonia uptake.

Table 5: The total heterotrophic counts (THC) recorded in the model brackish water systems with different C/N ratios amended with glucose and urea

Days	Water (W) Sediment(S)	1:1	5:1	20:1	50:1	100:1
Initial	W	$4.3x10^2$	$3.8x10^2$	$3.2x10^2$	$4.2x10^2$	$3.9x10^2$
	S	$5.1x10^3$	$6.2x10^2$	$6.1x10^2$	$5.6x10^3$	$5.2x10^3$
7th day	W	$6.8x10^3$	$5.6x10^2$	$4.2x10^2$	$4.1x10^4$	$9.2x10^4$
	S	$3.6x10^4$	$4.9x10^3$	$5.9x10^3$	$9.5x10^5$	$3.3x10^5$
14th day	W	$5.6x10^4$	$4.7x10^3$	$5.8x10^4$	$6.5x10^4$	$3.6x10^5$
	S	$3.8x10^5$	$5.2x10^4$	$4.5x10^5$	$9.5x10^6$	$1.02x10^6$
21st day	W	$3.7x10^5$	$5.7x10^4$	$3.4x10^4$	$3.9x10^3$	
	S	$4.1x10^6$	$3.7x10^5$	$3.5x10^5$	$5.6x10^2$	-
28th day	W	$5.3x10^3$	$6.2x10^3$	$4.7x10^4$	-	-
	S	$3.8x\ l0^4$	$4.6x10^4$	$3.9x10^5$	-	-
35th day	W	$7.1x10^3$	$5.1x10^2$	$3.9x10^3$	-	-
	S	$4.6x10^4$	$6.3x10^3$	$6.1x10^4$	-	-
42nd day	W	$3.6x10^2$	$3.5x10^2$	$3.5x10^2$	-	-
	S	$5.3x10^3$	$5.7x10^3$	$5.7x10^3$	-	-
49nd day	W	$3.3x10^2$	$4.3x10^2$	$6.8x10^2$	-	-
	S	$3.9x10^l$	$3.5x10^3$	$4.2x10^3$	-	-
56th day	W	$3.1x10^2$	$3.6x10^2$	-	-	-
	S	$3.1x10^2$	$3.4x10^3$	-	-	-

Table 6: The total heterotrophic counts (THC) recorded in the model brackish water systems with different *C/N* ratios amended with sucrose

Days	Water (W) Sediment(S)	20:1	50:1	100:1
Initial	W	$4.1x10^2$	$3.8x10^2$	$4.3x10^2$
	S	$3.7x10^2$	$4.1x10^3$	$4.9x10^3$
7th day	W	$5.7x10^2$	$3.6x10^4$	$5.5x10^4$
	S	$4.9x10^4$	$7.5x10^5$	$6.2x10^5$
14th day	W	$4.3x10^5$	$5.0x10^5$	$1.29x10^5$
	S	$3.8x10^6$	$7.8x10^6$	$3.8x10^6$
21st day	W	$7.9x10^5$	-	-
	S	$4.1x10^6$	-	-
28th day	W	$6.3x10^3$	-	-
	S	$3.6x10^4$	-	-
35th day	W	$4.7x10^3$	-	-
	S	$3.2x10^4$	-	-
42nd day	W	-	-	-
	S	-	-	-
49nd day	W	-	-	-
	S	-	-	-
56th day	W	-	-	-
	S	-	-	-

Table 7. The total heterotrophic counts (THC) recorded in the model brackish water systems with different C/N ratios amended with starch

Days	Water (W) Sediment(S)	20:1	50:1	100:1
Initial	W	$4.6x10^2$	$3.8x10^2$	$4.6x10^2$
	S	$3.3x10^2$	$5.2x10^2$	$3.7x10^2$
7th day	W	$6.5x10^2$	$4.1x10^2$	$5.7x10^2$
	S	$3.6x10^3$	$5.6x10^3$	$6.1x10^2$
14th day	W	$4.8x10^3$	$4.8x10^2$	$5.3x10^2$
	S	$5.4x10^4$	$6.1x10^3$	$4.5x10^3$
21st day	W	$5.9x10^4$	$5.6x10^3$	$6.3x10^3$
	S	$7.6x10^5$	$4.3x10^3$	$5.6x10^4$
28th day	W	$8.2x10^4$	$7.8x10^4$	$4.2x10^4$
	S	$4.3x10^4$	$8.9x10^5$	$6.4x10^5$
35th day	W	$3.9x10^3$	$3.9x10^2$	$3.9x10^2$
	S	$5.4x10^3$	$5.6x10^3$	$7.1x10^3$
42nd day	W	$6.9x10^2$	$4.1x10^2$	-
	S	$4.8x10^2$	$3.8x10^3$	-
49nd day	W	$3.3x10^2$	-	-
	S	$5.6x10^2$	-	-
56th day	W	-	-	-
	S	-	-	-

Table 8: The population of heterotrophic ammonia and nitrite oxidizers recorded in the water of model brackish water systems with different C/N ratios amended with urea

Days	**Ammonia oxidisers** **Nitrite oxidisers**	**1:1**	**5:1**
Initial	Ammonia oxidizers	3.30x10^{2}	3.70x10^{2}
	Nitrite oxidizers	5.40.x10^{2}	3.30x10^{2}
7th day	Ammonia oxidizers	9.60 x10^{2}	5.30x10^{2}
	Nitrite oxidizers	8.90 x10^{2}	1.51x10^{3}
14th day	Ammonia oxidizers	5.90 x10^{3}	7.30x10^{2}
	Nitrite oxidizers	3.70 x10^{3}	5.60x10^{3}
21st day	Ammonia oxidizers	1.29 x10^{3}	4.10x10^{3}
	Nitrite oxidizers	5.60 x10^{3}	3.40x10^{3}
28th day	Ammonia oxidizers	2.86 x10^{4}	6.90x10^{4}
	Nitrite oxidizers	4.60 x10^{2}	5.90x10^{5}
35th day	Ammonia oxidizers	7.30 x10^{3}	4.30x10^{2}
	Nitrite oxidizers	9.70 x10^{2}	6.10x10^{3}
42nd day	Ammonia oxidizers	2.43 x10^{3}	3.20x10^{2}
	Nitrite oxidizers	3.70 x10^{2}	4.90x10^{3}
49nd day	Ammonia oxidizers	1.16 x10^{3}	4.10 x10^{2}
	Nitrite oxidizers	3.40 x10^{2}	5.70 x10^{2}
56th day	Ammonia oxidizers	5.60 x10^{2}	3.90 x10^{2}
	Nitrite oxidizers	5.90 x10^{2}	3.10 x10^{2}

Table 9: The population of heterotrophic ammonia and nitrite oxidizers recorded in the water of model brackish water systems with different C/N ratio amended with glucose

Days	**Ammonia oxidisers** **Nitrite oxidisers**	**20:1**	**50:1**	**100:1**
Initial	Ammonia oxidizers	4.20x10^{2}	3.90x10^{2}	3.60x10^{2}
	Nitrite oxidizers	3.30x10^{2}	3.40x10^{2}	3.10x10^{2}
7th day	Ammonia oxidizers	9.70x10^{2}	5.70x10^{2}	5.60x10^{2}
	Nitrite oxidizers	7.90x10^{2}	4.90x10^{2}	6.30x10^{2}
14th day	Ammonia oxidizers	1.39x10^{3}	4.60x10^{2}	4.30x10^{2}
	Nitrite oxidizers	2.71x10^{3}	8.90x10^{2}	5.30x10^{2}
21st day	Ammonia oxidizers	4.20x10^{2}	3.60 x10^{2}	-
	Nitrite oxidizers	7.10x10^{2}	3.80 x10^{2}	-
28th day	Ammonia oxidizers	3.50x10^{2}	-	-
	Nitrite oxidizers	4.50x10^{2}	-	-
35th day	Ammonia oxidizers	3.10x10^{2}	-	-
	Nitrite oxidizers	< 2	-	-
42nd day	Ammonia oxidizers	< 2	-	-
	Nitrite oxidizers	< 2	-	-
49nd day	Ammonia oxidizers	-	-	-
	Nitrite oxidizers	-	-	-
56th day	Ammonia oxidizers	-	-	-
	Nitrite oxidizers	-	-	-

Table 10: The population of heterotrophic ammonia and nitrite oxidizers recorded in water of model brackish water systems with different C/N ratios amended with sucrose

Days	**Ammonia oxidisers** **Nitrite oxidisers**	**20:1**	**50:1**	**100:1**
Initial	Ammonia oxidisers	4.50x10^{2}	3.70x10^{2}	4.90x10^{2}
	Nitrite oxidisers	3.50x10^{2}	3.20x10^{2}	6.40x10^{2}
7th day	Ammonia oxidisers	9.30x10^{2}	6.40x10^{2}	1.72x10^{3}
	Nitrite oxidisers	6.70x10^{2}	4.30x10^{2}	4.30x10^{2}
14th day	Ammonia oxidisers	1.13x10^{3}	4.60x10^{2}	6.20x10^{2}
	Nitrite oxidisers	5.20x10^{2}	5.60x10^{2}	3.10x10^{2}
21st day	Ammonia oxidisers	7.30x10^{2}	3.80 x10^{2}	< 2
	Nitrite oxidisers	3.90x10^{2}	4.30 x10^{2}	< 2
28th day	Ammonia oxidisers	4.20x10^{2}	4.20 x10^{2}	-
	Nitrite oxidisers	3.30x10^{2}	< 2	-
35th day	Ammonia oxidisers	3.90x10^{2}	-	-
	Nitrite oxidisers	< 2	-	-
42nd day	Ammonia oxidisers	-	-	-
	Nitrite oxidisers	-	-	-
49nd day	Ammonia oxidisers	-	-	-
	Nitrite oxidisers	-	-	-
56th day	Ammonia oxidisers	-	-	-
	Nitrite oxidisers	-	-	-

Table 11: The population of heterotrophic ammonia and nitrite oxidizers recorded in the water of model brackish water systems with different C/N ratios amended with starch

Days	Ammonia oxidizers Nitrite oxidizers	20:1	50:1	100:1
Initial	Ammonia oxidizers	4.30x10^{2}	5.90x10^{2}	4.60x10^{2}
	Nitrite oxidizers	3.20x10^{2}	4.30x10^{2}	3.40x10^{2}
7th day	Ammonia oxidizers	7.20x10^{2}	1.28x10^{3}	2.31x10^{3}
	Nitrite oxidizers	9.30x10^{2}	1.79x10^{3}	4.60x10^{2}
14th day	Ammonia oxidizers	6.10 x10^{2}	8.10 x10^{2}	5.20 x10^{2}
	Nitrite oxidizers	2.10x10^{3}	4.60x10^{2}	9.30x10^{2}
21st day	Ammonia oxidizers	7.30x10^{3}	7.40x10^{2}	4.10x10^{2}
	Nitrite oxidizers	5.90 x10^{2}	5.30 x10^{2}	3.60 x10^{3}
28th day	Ammonia oxidizers	5.90x10^{3}	7.10 x10^{2}	3.20x10^{2}
	Nitrite oxidizers	7.90x10^{2}	4.90 x10^{2}	4.10x10^{2}
35th day	Ammonia oxidizers	4.70x10^{2}	3.40 x10^{2}	5.70 x10^{2}
	Nitrite oxidizers	6.80x10^{2}	5.30 x10^{2}	3.40x10^{2}
42nd day	Ammonia oxidizers	5.60x10^{2}	4.30x10^{2}	3.90 x10^{2}
	Nitrite oxidizers	4.90 x10^{2}	3.90 x10^{2}	3.40x10^{2}
49nd day	Ammonia oxidizers	4.70 x10^{2}	4.10x10^{2}	3.30 x10^{2}
	Nitrite oxidizers	3.60 x10^{2}	3.10x10^{2}	3.10x10^{2}
56th day	Ammonia oxidisers	-	-	-
	Nitrite oxidisers	-	-	-

Table 12: Two - way ANOVA analysis of the highest concentration of ammonia registered in water with different C/N ratios in model brackish water systems amended with different carbon sources

Source of variation	Df	Sum of squares	Mean square	F-value	Level of significance
Between rows (C/N ratios)	3	6107561.00	2035854.00	13.625	P<0.05
Between Columns(Carbon amendments)	2	1669424.00	834712.00	5.587	N.S
Error	6	896494.00	149415.70		

Table 13: Two - way ANOVA analysis of the highest concentration of ammonia registered in sediment with different C/N ratios in model brackish water systems amended with different carbon sources

Source of variation	Df	Sum of squares	Mean square	F-value	Level of significance
Between rows (C/N ratios)	3	342114.30	114038.10	18.1011	P<0.05
Between Columns(Carbon amendments)	2	16445.00	8222.50	1.305	N.S
Error	6	37801.25	6300.21		

Table 14: Quantitative relationship existing between the highest level of ammonia and C/N ratios

Carbon source	Water (W)/ Sediment (S)	Semi-Log/ Log-lag	Df	Intercept (a)	Slope(b)	Correlation Coefficient (r)	Level of significance
Glucose	W	Log-log	2	3.093	-0.015	-0.9992	P<0.01
Sucrose	W	Log-log	2	3.147	-0.012	-0.9743	P<0.05
Starch	W	Semi log	2	3.393	-0.006	-0.9660	P<0.05
Glucose	S	Semi log	2	2.966	-0.008	-0.9739	P<0.05
Sucrose	S	Semi log	2	2.872	-0.003	-0.9760	P<0.05
Starch	S	Semi log	2	2.827	-0.004	-0.9706	P<0.05

Table 15: Quantitative relationship existing between concentration of nitrite and counts of heterotrophic nitrite oxidizers

C/N Ratios	Water (W)/ Sediment (S)	df	Intercept (a)	Slope (b)	Correlation Coefficient (r)	Level of significance
All ratios pooled	W&S	144	4.2	+90.8	0.8210	P<0.01
All ratios pooled	W&S	160	1.9	+14.76	0.8105	P<0.01

4. Discussion

In the present investigation the effect of C/N ratio in enhancing the heterotrophic activity was investigated by using three different sources of carbon. To ascertain the impact of C/N ratio on the heterotrophic activity of aquatic ecosystems mixture of sediment and water (brackish water) was used as the laboratory model to stimulate the sediment water interactions that decide microbial activities in aquatic ecosystems.

When compared to water, sediment would support higher biomass of heterotrophs due to its comparatively higher surface area [19, 20]. Superficial layer of sediment had higher biomass and activities of heterotrophs than the sub-surface layers and a depth of 10cm the heterotrophic population [41] would be approximately 1% of that present in surface layer. In the present investigation depth of the sediment was never permitted to exceed 1cm and the sediment-water mixture was thoroughly aerated to effect aerobic condition throughout sediment and water. However, in brackish water models despite the efforts taken anaerobic conditions set-in following the prevalence of denitrification and sulphate reduction owing to the imbalance caused between oxygen supply and uptake rate.

C: N ratio of 5:1 has been accepted as the ideal ratio for the proliferation of heterotrophs both in water and sediments. The importance of this ratio in deciding the heterotrophic population and activity could be attributed to the stoichiometric composition of microbes which can be represented as $C5H7N02$ [1, 13]. However, in the brackish water both in water and sediments the highest ammonia concentration was observed to be influenced by C/N ratio with least influence of the type of organic carbon source used for amendment

In the present study, in brackish water increase in C/N ratio was observed to enhance bacterial multiplication and the highest heterotrophic activity was recorded in C/N ratio of 100:1. Unlike in fresh water, in brackish water, the optimal C: N ratio for boosting heterotrophic activity is yet to be established. In brackish water apart from the ratio, the type of carbon source used to enhance C: N ratio also seems to play a veritable role in deciding the activity and population of heterotrophs [10]. It is a well-accepted phenomenon that nitrogen occurs as the limiting nutrient in most of the brackish water ecosystems [16, 36]. Perhaps, heterotrophs living under such unique nitrogen limiting conditions may have low requirement of nitrogen and hence high C/N ratio may accelerate their production rate. However, further information is required to provide solid information in this regard.

The rate of ammonia uptake recorded in different C/N ratios amended with different sources of carbon showed that the ammonia in brackish water was totally eliminated from the water and sediment even prior to the termination of the experiments.

In the case of brackish water, extensive utilization of ammonia was followed by sulphate reduction and consequent hydrogen sulfide production [30]. Production of hydrogen sulfide in higher C/N ratios especially in carbon amendments with glucose and sucrose happened primarily due to the fact that the oxygen demand imparted by the multiplying heterotrophs exceeded that the oxygen supply rate effected with mechanical aeration. Comparatively lower oxygen holding capacity of brackish water than that of freshwater assumes importance in this regard. It deserves mention here that sulphate reduction should have been initiated only after the completion of denitrification [9]. Thus the necessity of brackish water sediment and water to turn anaerobic under heavy oxygen demand remains justified. Ammonia which is known to be present even anaerobic condition was totally absent in brackish water systems under conditions of high C/N ratios. This observation confirmed that ammonia was totally stripped off from sediment and water prior to the system becoming anaerobic with hydrogen sulfide production. As observed in the case of ammonia, nitrate was also observed to be removed by the accelerated heterotrophic activity and the enhanced biomass.

A thorough knowledge is needed in this regard for brackish water ecosystems which are well known for their nitrogen impoverished condition. In the present study, in the water of all the model systems population of heterotrophic nitrite oxidizers showed highly significant ($P<0.01$) positive correlation with the level of available nitrite. This sustained operation of nitrite oxidation resulted in nitrate production, which in turn was assimilated by the nitrogen requiring heterotrophs. Thus, ammonia as such or in nitrified condition as nitrate was stripped off from the water by the heterotrophs to synthesize body protein.

Results obtained in nutrient spiking experiments confirmed that phosphate did not occur as the limiting nutrient in any of the treatments. Biochemically important trace metals such as iron, manganese, molybdenum zinc, and cobalt also did not limit the population of heterotrophs. However, the anticipated limiting activity following sulphate reduction warrants mention here. Following hydrogen sulphide production, soluble inorganic chemical species of these metals react with hydrogen sulphide to produce insoluble metallic sulphides. Thus, it was explicit that only under such conditions trace metals may impart limiting activity over heterotrophic population. Generally, ammonia injection triggered ammonia utilization by heterotrophs.

Indicating higher demand for ammonia than that for phosphate and trace metals. The turnover of microbial biomass on soil amendment with glucose, urea, phosphate and mineral nutrients and the available nitrogen and trace metals might inhibit bacterial population in terrestrial conditions [22].

In aquaculture systems, ammonia and nitrite pose problem to finfish and shellfish under culture. Usually, with increase in concentration of ammonia in the water the rate of excretion of ammonia by fishes decreases with elevation in the level of ammonia in blood and other tissues. This elevated blood pH adversely affects enzyme catalyzed reactions and membrane stability. Generally, ammonia toxicity increases oxygen consumption by tissues, damages gills and restricts the oxygen carrying capacity of the blood. Nitrite toxicity results in the production of methaemoglobin in fishes which is commonly known as brown blood disease. Since, methaemoglobin is not an effective oxygen carrier; sustained absorption of nitrite by fishes may lead to hypoxia and cyanosis. Owing to the above furnished facts, high concentration of ammonia and nitrite in fish culture systems may create toxic conditions or weaken the organism to become vulnerable to disease [17, 13, 30, 32].

As observed in the present investigation, accelerated rate of ammonia absorption by heterotrophs will restrict ammonia oxidation in high C/N ratios without inhibiting the oxidation of nitrite to nitrate. Such conditions of high C/N ratios offer much scope for using saccharides in disease prevention practices.

To conclude, enhancement of C/N ratios with suitable carbon source may control the concentration of ammonia and nitrite in aquaculture systems as well as transform them into bacterial protein of high food value [4, 30]. It is needless to emphasize the role of basic food in semi-intensive culture systems. The present investigations found that starch when used for carbon amendment behave in a different way when compared to sucrose and glucose. Starch did not effectively supply the carbon needed but in turn would release the biologically assimilable carbon through co metabolism by heterotrophs [38]. This sustained slow reaction results in the gradual depletion of ammonia and nitrate from the water. In brackish water, starch application did not trigger sulphate reduction immediately after application. Perhaps, a lower dose of starch with C/N ratio of 20: 1 may be more effective in improving water quality characteristics in shrimp culture systems. Chances are bright to believe that in future, starch may play a vital role in improving disease free conditions in shrimp culture systems.

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